

WHAT IS CLAIMED IS:

1. A method for obtaining a cell wall C-polysaccharide antigen containing not more than about 10% protein from the bacterium *Streptococcus pneumoniae* which comprises the steps of:

- (a) culturing the bacterium for a time requisite to obtain a sample of desired size and harvesting the bacterial cells therefrom in the form of a wet cell pellet;
- (b) suspending the wet cell pellet in an alkaline solution and mixing;
- (c) adjusting the pH to an acid pH with a strong acid and centrifuging;
- (d) separating the supernatant from step (d) and adjusting its pH to approximate neutrality;
- (e) digesting this product with a broad spectrum protease enzyme preparation to destroy residual proteins;
- (f) adjusting the pH to the alkaline side with a weakly alkaline aqueous solution
- (g) separating out the essentially protein free carbohydrate or polysaccharide antigen on a size exclusion column equilibrated with a weakly alkaline solution; and
- (h) pooling material eluted in the first peak and adjusting its pH to approximate neutrality.

2. The cell wall C-polysaccharide antigen containing not more than about 10% protein obtained by the method of claim 1.

3. A method according to claim 1 in which the alkaline solution of step (b) comprises about 20 ml. per gram of said wet cell pellet of 0.1M aqueous sodium hydroxide.

4. A method according to claim 1 in which in step (c) the pH is adjusted to about 3.0.

5. A method according to claim 1 in which, in step (f) the pH is adjusted to a pH between about 10 and about 11.

6. A method according to claim 1 in which, after step (h), a lyophilization step is performed.

7. A method for the purification of raw antibodies to *S. pneumoniae* which comprises the steps of:

- (a) separating from *S. pneumoniae* bacteria a cell-wall C-polysaccharide antigen containing not more than about 10% protein;
- (b) conjugating said antigen to one end of a two-ended spacer molecule to form a conjugate of said antigen with the spacer molecule;
- (c) coupling the conjugate to an activated chromatographic column;
- (d) subjecting said raw antibodies to affinity chromatography on said column from step (c) to obtain purified antigen-specific antibodies;
- (e) eluting from said column the purified antigen-specific antibodies.

8. Purified antigen-specific antibodies to the cell wall C-polysaccharide of *S. pneumoniae* obtained by the method of claim 7.

9. A chromatographic column for affinity purification of raw antibodies to *S. pneumoniae* having coupled thereto by a spacer molecule a purified C-polysaccharide cell wall antigen of *S. pneumoniae* containing not more than about 10% protein.

10. A method of assaying for the presence of *S. pneumoniae* or its cell-wall C-polysaccharide antigen in a fluid, which method comprises the steps of:

- (a) extracting from a culture of *S. pneumoniae* bacteria the cell wall C-polysaccharide antigen thereof containing not more than about 10% protein,
- (b) coupling said antigen to a spacer molecule to form a conjugate,

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- (c) coupling the conjugate from step (b) to a chromatographic affinity column,
- (d) purifying raw antibodies to *S. pneumoniae* bacteria with the chromatographic affinity column of step (c) to produce purified antigen-specific antibodies; and
- (e) using the purified antibodies of step (d) to detect the presence or absence of *S. pneumoniae* or its α -polysaccharide cell wall antigen in a fluid.
11. The method of claim 10 in which the spacer molecule of step (b) is a protein molecule.
12. The method of claim 10 in which the spacer molecule of step (b) is a conjugate of hydrazine and bovine serum albumin.
13. The method of claim 10 wherein the fluid of step (e) is a natural fluid of mammalian origin.
14. The method of claim 13 wherein the fluid is human urine.
15. The method of claim 13 in which the fluid is obtained from a patient exhibiting clinical signs of a pneumonia-type illness.
16. The method of claim 15 in which step (e) is an immunoassay process.
17. The method of claim 15 in which step (e) is an immunochromatographic ("ICT") assay process.
18. The method of claim 17 wherein a portion of the purified antigen-specific antibodies from step (d) are conjugated to a labeling agent known to display a visible color when said antibodies react with the corresponding antigen.
19. The process of claim 18 wherein the labeling agent is finely divided metallic gold.

20. An ICT assay for the detection of *S. pneumoniae* bacteria or the C-polysaccharide cell wall antigen of said bacteria which comprises:

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- (a) contacting a sample of a fluid suspected of containing said bacteria or their antigen with an ICT device comprising a strip of a bibulous material, which strip has
 - (i) a first zone in which has been embedded a conjugate of
 - (1) a labeling agent that displays a visible color change upon reaction of antibodies with their corresponding antigenic binding partner and
 - (2) purified antigen-specific antibodies to the C-polysaccharide cell wall antigen of *S. pneumoniae*, said antibodies having been purified by passage over a chromatographic affinity column to which is conjugated a purified C-polysaccharide cell wall antigen of *S. pneumoniae* containing not more than about 10% protein
 - (ii) a second zone having bound thereto the same purified antigen-specific antibodies in unconjugated form, which zone is equipped with a window for viewing color changes,
 - (b) allowing said sample to flow laterally along said test strip to said first zone,
 - (c) allowing said sample together with said conjugate of antigen-specific antibodies and label, to flow laterally along said test strip to said second zone, and
 - (d) within approximately 15 minutes from the commencement of step (a) observing through said window whether a line of color has appeared in said second zone, thereby indicating the presence in the sample of *S. pneumoniae* or its cell wall C-polysaccharide antigen.

21. The method of claim 20 wherein the fluid suspected of containing *S. pneumoniae* bacteria or their C-polysaccharide cell wall antigen is a natural fluid of mammalian origin.

22. The method of claim 21 in which the natural fluid of mammalian origin is human urine.

23. The method of claim 21 in which the natural fluid of mammalian origin is blood or serum.

24. The method of claim 21 in which the natural fluid of mammalian origin is cerebrospinal fluid.

25. The method of claim 22 in which the labeling agent that displays a visible color change upon reaction of antibodies with their corresponding antigenic binding partner is a finely divided metal.

26. The method of claim 25 wherein the labeling agent is finely divided gold.

27. The method of claim 20 in which the labeling agent is finely divided gold and the fluid is human blood or serum.

28. The method of claim 20 in which the labeling agent is finely divided gold and the fluid is human cerebrospinal fluid.

29. The method of claim 20 in which the labeling agent is finely divided gold and the fluid is human urine.

30. An ICT device for the detection of *S. pneumoniae* or its C-polysaccharide cell wall antigen which comprises a strip of bibulous material having

- (i) a first zone in which has been embedded a conjugate of (1) a labeling agent that displays a visible color change upon the reaction of antibodies with their corresponding antigenic binding partner and (2) purified antigen-specific antibodies to the C-polysaccharide cell wall antigen of *S. pneumoniae*, said antibodies having been purified by passage over a chromatographic affinity column to which is conjugated a purified C-polysaccharide cell wall antigen of *S. pneumoniae* containing not more than about 10% protein,

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- (ii) a second zone having bound thereto the same purified antigen-specific antibodies in unconjugated form, which zone is equipped with a window for viewing color changes.

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